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Drug Metabolism in Man and Its Relationship to That in Three Rodent Species: Monooxygenase, Epoxide Hydrolase, and Glutathione S-Transferase Activities in Subcellular Fractions of Lung and Liver[1]

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Lipophilic drugs require metabolic conversion to hydrophilic derivatives to allow excretion. During this metabolism reactive intermediates causing mutagenic, carcinogenic, and other toxic effects may be generated. Reactive intermediates often can be inactivated by further metabolism. Usually, monooxygenases (MO)[3] conduct the first steps of metabolism of lipophilic xenobiotics. They mostly are the enzymes which produce a reactive intermediate (1–8). Glutathione S-transferases (GST) and, if the reactive intermediate is an epoxide, epoxide hydrolases (EH) are the enzymes which most frequently directly inactivate the reactive intermediate (4–6,9–12). Both enzymes, however, are sometimes also involved in the activation of xenobiotics, e.g., GST in the case of some 1,2-dihalogenated compounds (13), EH in the case of some polycyclic aromatic hydrocarbons (PAH) which possess an angular structure (14–18).

In vitro studies on drug metabolism use mostly preparations from liver, the organ with the highest capability for drug metabolism. Toxic effects frequently occur in extrahepatic tissues such as the lung. This, for instance, is the case with PAH, which are far more carcinogenic in the lung than in the liver. Knowledge of the activities of important drug metabolizing

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[3] Abbreviations: EH, epoxide hydrolase; GST, glutathione S-transferase; MO, monooxygenase; PAH, polycyclic aromatic hydrocarbons.

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enzymes in target organs in animals and man will help to understand susceptibilities of some organs of laboratory animals to certain agents and to extrapolate these data to man. The activities may be compared with activities of the liver, because a large number of studies on drug metabolism by liver enzymes is available and the organ is of primary importance for the metabolism of many foreign compounds and also because this organ often may compete with the target organ for metabolism of the xenobiotic.

In the present study, specific MO, EH, and GST activities were investigated in liver and lung from man and three laboratory animals. Since MO is a predominantly microsomal enzyme system with only low activities in other cellular membrane fractions and no detectable activity in the cytosolic fraction (19), it was determined in microsomal preparations. EH is located in membrane (4,19) and cytosolic fractions (20–22) in at least two different forms. We determined the microsomal EH, because the cytosolic EH has much lower specific activity for all those epoxides derived from PAH which have been tested to date (21). GST activities toward all substrates investigated so far are several fold higher in the cytosolic fraction than in membrane fractions (9,23) and were therefore measured in the cytosolic fraction. Because these enzymes may occur in different forms with different substrate specificities, substrates which are metabolized by a broad spectrum of different enzyme forms were employed with GST and MO (24,25). As to the latter, different forms may not only vary in the rate of metabolism, but also in the region of the xenobiotic molecule attacked (26–28). Inhibition by diagnostic inhibitors (25) was used as a first distinction between different groups of MO forms. Numerous data are already available, scattered throughout the literature, especially on monooxygenase and epoxide hydrolase activities in human and rat liver microsomes (29–48). The present study complements the available information with data on aspects which have so far received little attention, such as EH and GST in human lung and any of the investigated enzyme activities in the liver and lung of the hamster, a species frequently used in toxicological investigations. Moreover, a major purpose of this study was to obtain strictly comparable data on the total set of the investigated enzyme activities elaborated within a single study, since the values of specific activities of the investigated enzymes can markedly vary between studies performed in different laboratories.

MATERIALS AND METHODS

Chemicals. 7-Ethoxycoumarin was prepared by standard procedure as described (49). [^{3}H]Benzo[*a*]pyrene 4,5-oxide was synthesized according to the method of Dansette and Jerina (50) under conditions as described (51) and had a specific activity of 1.2 mCi/mmole. 2,4-Dinitrochlorobenzene

was bought from Sigma Chemie, Taufkirchen, West Germany, and recrystallized.

Animals. Adult male animals were used throughout. Sprague–Dawley rats (200–250 g) were obained from Versuchstieranstalt Wiga, Sulzfeld, West Germany, Syrian golden hamsters (100–140 g) from a private breeder. MNRI mice (15–20 g) were a gift from Dr. E. Pfeiffer from the Institute of Hygiene, University of Mainz, West Germany. Animals were kept under standardized conditions as described (52).

Human tissues. A survey on the patients included in this study is shown in Table 1.

Needle biopsy specimens of human liver were obtained for histological investigation. An aliquot (10–20 mg) of each sample was reserved for biochemical studies. Subcellular preparation was carried out promptly and fractions were lyophilized and stored at −25°. Lung samples were obtained during surgery, frozen immediately in liquid nitrogen, and kept at −70° until preparation.

Three of the liver biopsy donors were smokers and influences on hepatic enzyme activities cannot be excluded. Results on this point were contradictory. Prough *et al.* (53) could not observe a correlation between smoking habits and human liver benzo[*a*]pyrene MO levels, whereas Boobis and co-workers (36) found an increase in this enzyme in smokers which just reached statistical significance. In the present study enzyme levels of smokers did not differ from average values. In lung cells, induced MO activities were shown to revert to leves of nonsmokers within 3 months after cessation of smoking (54). Therefore in this study patients who had not smoked for at least 6 months were classified as nonsmokers.

Preparation of subcellular fractions. Animals were killed by cervical dislocation. Livers and lungs were removed and put in ice-cold 1.15% KCl containing 10 mM K-phosphate buffer, pH 7.4. Each organ was prepared separately. The organs were freed from adhering tissues. Human lung samples were warmed up rapidly to 0°C. All subsequent steps of preparation were performed at 0–4°C. Tissues were minced and homogenized in about 3 vol of 1.15% KCl containing 10 mM K-phosphate buffer, pH 7.4. The human lung samples were too fibrous for homogenization by the Potter–Elvehjem procedure which is normally used as standard procedure for homogenization of rodent lung and liver. Lungs of all species were therefore homogenized with an Ultra Turrax homogenizer (three times 10 sec interrupted by cooling in an ice bath for 1 min); livers of all species were homogenized with a Potter–Elvehjem all glass homogenizer (10 strokes). Potential influences of the different homogenization methods on specific enzyme activities were tested with both tissues from rat where the lung was sufficiently soft to be suitable for homogenization by either of the two methods. Both procedures gave tissue fractions with

TABLE 1

DETAILS OF THE DONORS OF LIVER AND LUNG BIOPSIES

Sample	Clinical diagnosis	Sex[a]	Age (years)	Exogenous influences[b]	Enzymes measured[c]
Liver 1	Cholelithiasis	F	27	NS	EH, GST
Liver 2	?	?	?	?	MO, GST
Liver 3	Cholelithiasis	F	32	NS	MO, EH, GST
Liver 4	Polyglobulia	M	52	NS	MO, EH
Liver 5	Suppurative pneumonia	M	45	S	MO, GST
Liver 6	Diffuse goiter	M	28	NS	MO, EH
Liver 7	Prostration	M	43	NS	EH
Liver 8	Cutaneous sarcoidosis	F	53	NS, Fluorcortolone (5 days)	MO, EH
Liver 9	Hodgkin lymphoma	M	41	S	MO, EH
Liver 10	Non Hodgkin lymphoma	M	66	NS, Methyldigoxin (11 days), Verapamil (11 days)	MO, EH, GST
Liver 11	Hodgkin lymphoma	M	41	NS, Promethazine	MO, EH, GST
Liver 12	Hodgkin lymphoma	M	22	S	MO, EH, GST
Liver 13	Hodgkin lymphoma	F	64	NS, Ethyldigoxin (20 days) Hydrochlorothiazide (13 days)	GST
Liver 14	Pyrexia	?	?	NS	GST
Liver 15	Non Hodgkin lymphoma	M	54	NS	GST
Lung 1	Bronchiectasis	F	49	NS	MO, EH, GST
Lung 2	Mediastinal cyst	M	39	NS	MO, EH, GST
Lung 3	Pleurodesis	F	32	NS	MO, EH, GST
Lung 4	Spontaneous pneumothorax	M	48	NS	MO, EH, GST
Lung 5	Aortal aneurysm	M	63	NS, Dipyridamole (28 days)	MO, EH, GST
Lung 6	Thoracal cyst	M	55	NS, Amidopyrine (28 days)	MO, EH, GST
Lung 7	Emphysemal vesicle	M	67	NS	MO, EH, GST
Lung 8	Diaphragmatic hernie	M	52	NS	MO, EH, GST
Lung 9	Bronchiectasis	F	43	NS	MO, EH, GST
Lung 10	Tuberculoma	M	50	NS	MO, EH, GST

Lung 11	Relapsed pneumonia	M	81	NS	MO, EH, GST
Lung 12	Thoracal cyst	M	56	NS	MO, EH, GST

Notes. Enzyme activities were determined in liver needle biopsies which primarily were taken for diagnostic purposes and in lung samples from patients who underwent thoracal surgery. The liver specimens used did not show any pathological alterations at histological examination. Serum parameters (bilirubin concentration and activities of γ-glutamyl transferase and transaminases) displayed values of normal, healthy subjects. The lung samples were from patients not suffering from pulmonary tumors. None of the patients investigated used more than 50 g alcohol per day and none was on long-term treatment with drugs known to be inducers of the enzymes measured. Other pharmaceuticals used during the last 4 weeks prior to biopsy and smoking habits are listed in the table. Pharmaceuticals were given in conventional doses for the number of days indicated in brackets.

[a] F, female; M, male.

[b] NS, nonsmoker (abstinence for at least 6 months prior to biopsy); S, smoker (at least 10 cigarettes per day up to at least the third last day before biopsy).

[c] Due to lack of sufficient material the three enzymes could not be determined in each liver sample.

similar specific activities of the enzymes investigated. Deviations from the mean value were less than 20% for the microsomal and less than 10% for the cytosolic enzymes. The homogenates were centrifuged at 10,000 *g* for 15 min and the resulting supernatant fractions were centrifuged at 100,000 *g* for 1 hr to give the cytosolic fraction and the microsomal pellet. Microsomes were washed by resuspending in buffered KCl and recentrifuging at 100,000 *g* for 30 min. Washed microsomes were resuspended in the same medium. Due to the low amounts of tissue available, the slightly modified micromethod was used with human liver biopsies as previously published (32). Microsomal suspensions and 100,000 *g* supernatant fractions were freeze dried and stored under vacuum at −25°C.

Enzyme assays. The O deethylation of 7-ethoxycoumarin was measured with the direct fluorimetric assay described by Ullrich and Weber (55) using three diagnostic *in vitro* inhibitors for crude differentiation of cytochrome P-450 forms (25). The assay was modified for human lung microsomal preparations where extremely low enzyme activities exist. Cofactor concentrations in the assay mixture were altered because both NADPH and umbelliferone fluoresce at 460 nm when excited at 385 nm. We reduced the NADPH amount within the range of saturating concentrations in order to diminish the resulting fluorescence quenching. Optimal cofactor concentrations in the assay mixture were 4×10^{-5} M NADPH, 2×10^{-5} M NADH, 3×10^{-3} M glucose-6-phosphate, and 0.56 U glucose-6-phosphate dehydrogenase per incubation. EH was assayed radiometrically using [^{3}H]benzo[*a*]pyrene 4,5-oxide as substrate (51). GST activities were determined according to the method of Habig *et al.* (56) with 2,4-dinitrochlorobenzene as substrate. The assay mixture was incubated at 37°C and had a pH value of 6.5. All incubations were performed in duplicates and at least at two different protein concentrations. Protein concentrations were determined by the method of Lowry *et al.* (57). Linearity with respect to protein concentration and incubation time was ascertained for all samples.

RESULTS

Stability of Enzymes in Human Liver and Lung Preparations

Lyophilization of human liver subcellular fractions or freezing and thawing of human lung samples had no significant (<15%) effect on the specific activities of the investigated enzymes. Enzyme activities in the lyophilized liver subcellular fractions proved to be stable for at least 4 weeks when kept at −25°C. Human lung samples stored at −70°C showed no measurable loss of enzyme activities over at least 1 year. In freshly prepared subcellular fractions of this tissue kept ice cold, the enzyme

stabilities were in the sequence MO, EH, GST, in that no measurable decrease in activity was observable for 4, 24, and at least 72 hr, respectively. Therefore, assays in human lung fractions were performed promptly after preparation in this order.

Enzyme Activities in Liver Preparations

Using the broad spectrum substrate 7-ethoxycoumarin among the four species tested, human liver microsomes showed the lowest specific MO activity, followed by preparations from rat, mouse, and hamster, which were approximately two-, four-, and sixfold more active (Table 2). Microsomal EH activity toward benzo[*a*]pyrene 4,5-oxide as substrate was lowest in mouse, while man, hamster, and rat showed a similar about

TABLE 2
SPECIFIC ENZYME ACTIVITIES IN SUBCELLULAR PREPARATIONS OF RODENT AND HUMAN ORGANS

Species	Organ	Number of individuals[a]	Monooxygenase	Epoxide hydrolase	Glutathione S-transferase
			(nmole product/min/mg protein)		
Man	Liver	15	0.418 ± 0.157	8.24 ± 2.41	1650 ± 480
	highest		0.732	11.70	2570
	lowest		0.156	4.76	1060
	Lung	12	0.0006 ± 0.0003	0.74 ± 0.20	78 ± 47
	highest		0.0013	1.21	156
	lowest		< 0.0001[b]	0.41	18
Sprague–Dawley rat					
	Liver	5	0.814 ± 0.118	8.51 ± 0.32	1380 ± 110
	Lung	5	0.111 ± 0.035	0.51 ± 0.05	77 ± 5
Syrian golden hamster					
	Liver	5	2.570 ± 0.580	9.72 ± 0.98	4200 ± 110
	Lung	5	0.164 ± 0.042	2.27 ± 0.15	325 ± 25
NMRI mouse					
	Liver	5	1.760 ± 0.115	1.96 ± 0.22	5290 ± 430
	Lung	5	0.732 ± 0.115	0.52 ± 0.10	727 ± 64

Notes. Activities of monooxygenase with 7-ethoxycoumarin and epoxide hydrolase with benzo[*a*]pyrene 4,5-oxide as substrates were determined in microsomes, glutathione S-transferase activity with 2,4-dinitro-chlorobenzene in the cytosolic fraction. Values are means ± SD of the activities determined in the given number of individuals.

[a] For lack of sufficient protein in human liver biopsies the activities of each enzyme were measured in 10 individuals among the 15 patients (see Table 1).

[b] Human lung specific MO activity was not above the blank in three patients.

fourfold higher specific activity than did the mouse. Using the broad spectrum substrate 2,4-dinitrochlorobenzene specific GST activity was similarly low in rat and man, whereas hamster and mouse had threefold and 3.5-fold activities, respectively. Thus, for all three enzyme activities investigated, rat liver contained specific activities very similar to those of man. In contrast, mouse and hamster displayed substantially higher MO and GST activities. In the mouse, furthermore, specific EH activity was considerable lower than in the other three species.

Interindividual variations of the enzyme activities were larger in man than in the laboratory animals, but the highest MO and GST activities among the individual subjects tested were all below the activities in hamster and mouse liver and lung (Table 2).

Enzyme Activities in Lung Preparations

The most striking result was the much lower MO activity in human than in rodent lung preparations (Table 2). Rat and hamster showed a 180- and 270-fold higher activity than the mean activity observed in human preparations. In mice the specific activity was 1200-fold higher. In contrast to MO, differences in specific EH activities among the species were small. In rat and mouse lung EH activity was marginally lower, in hamster it was threefold higher than in man. Among the four species tested, man and rat showed similar specific GST activity in lung cytosol, whereas the activities in hamster and mouse were four- and ninefold higher. Thus, with respect to all investigated enzyme activities in the lung, rat among the rodents studied was closest to man. EH and GST activities were very similar in rat and man. MO was much higher in rat than in man, but still lower than in the other two rodent species.

A comparison of the enzyme activities in lung and liver (Table 3) shows that the specific activity of any enzyme in any species was lower

TABLE 3
COMPARISON OF SPECIFIC ENZYME ACTIVITIES IN LIVER AND LUNG

Species	Monooxygenase	Epoxide hydrolase	Glutathione S-transferase
Man	697	11	21
Sprague–Dawley rat	7	17	18
Syrian golden hamster	16	4	13
NMRI mouse	2.4	4	7

Notes. Values show ratios between the specific activities in liver and lung subcellular preparations. The absolute specific activities used for this calculation are shown in Table 2. Substrates were 7-ethoxycoumarin (monooxygenase), benzo[*a*]pyrene 4,5-oxide (epoxide hydrolase), and 2,4-dinitrochlorobenzene (glutathione S-transferase).

in lung than in liver. The ratio of hepatic and pulmonary specific activity was lowest in mouse with all three enzymes. In the other species, all ratios between liver and lung activity with one exception varied between 4 and 21. In man, liver showed a much higher MO activity than lung due to the extremely low activity in microsomes of the latter organ.

Effects of Compounds Which Preferentially Inhibit Distinct Monooxygenase Forms

Some compounds strongly inhibit certain forms of MO *in vitro* while at the same concentrations they do not greatly affect other forms. They therefore may be employed to characterize MO patterns. In the present study we used metyrapone, tetrahydrofuran, and α-naphthoflavone, compounds known to inhibit those MO forms inducible by phenobarbital, ethanol, and 3-methylcholanthrene, respectively, with remarkable selectivity (25). Two of them, metyrapone and α-naphthoflavone, have also been shown to differentially inhibit MO forms in human liver microsomes (40). A comparison of the effects of these inhibitors in lung and liver in the different species indicates that inhibition patterns differed much more between the two organs than they did among the species (Fig. 1). In the liver of all four species the largest degree of inhibition was brought about by the standard concentration (10^{-2} M) of tetrahydrofuran. The standard concentration (10^{-5} M) of metyrapone also decreased the MO activity in all liver preparations albeit to a lesser extent. In lung the relative degree of inhibition by the standard concentrations of metyrapone and tetrahydrofuran was reversed compared to liver. Metyrapone was the most potent MO inhibitor in the lung of all four species while tetrahydrofuran had a weaker effect on the MO activity. Both in lung and liver the change

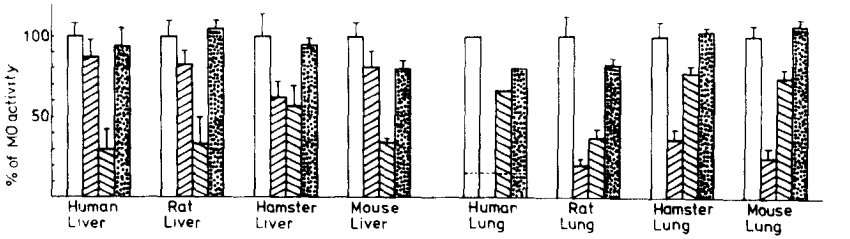

FIG. 1. *In vitro* inhibition of 7-ethoxycoumarin *O*-deethylase in rodent and human microsomal fractions from liver and lung. Bars display microsomal 7-ethoxycoumarin *O*-deethylase activities in the presence of 1×10^{-5} M metyrapone (▨), 1×10^{-2} M tetrahydrofuran (▧), and 2×10^{-5} M α-naphthoflavone (▩) as percentage of the activities in the absence of an inhibitor (□). Values are means ± SD of determinations in liver preparations from five animals of each rodent species and seven human individuals. Inhibition in lung microsomes was studied in a single human subject and in five animals from each rodent species. The interrupted line marks detection limit for 7-ethoxycoumarin *O*-deethylase.

of activity by α-naphthoflavone was less than or equal to 20%. In the liver α-naphthoflavone slightly increased the activity with the rat and slightly decreased the activity with the other species. In lung it slightly enhanced hamster and mouse MO activity and reduced it in preparations from man and rat.

DISCUSSION

The enzymes investigated in the present study play an important role in the formation of reactive intermediates from various xenobiotics and in the detoxification of these metabolites (1–12). Some problems arise from the observation that many drug metabolizing enzymes exist in multiple forms with different substrate preferences. This may least be the case with microsomal EH. Although some experiments may indicate the existence of several forms of microsomal EH in the same species (35), these forms only slightly differed in catalytic properties. Differences in substrate preference among EH from various mammalian species were found, but were not very great, especially not among various rodent species (11,33,58–60). Recently, firm evidence for the existence of at least one additional form of microsomal EH with a radically different substrate specificity has been obtained (61,62). However, this novel form ("EH_{ch}") has a very narrow substrate specificity for some steroid 5,6-epoxides and, so far, not a single xenobiotic epoxide was accepted as a substrate by this form. In contrast to EH, the existence of multiple forms of MO and GST with broadly overlapping yet substantially different substrate specificities is well documented (9,26–28,63–65). Evidence has been obtained that in human liver there is more than one form of MO present (34,36,66). For this reason we used substrates which are readily metabolized by a variety of enzyme forms. Thus our results may be representative for many substrates, although the relative metabolic rates in the different species of a substrate which is metabolized to a predominant extent by a single enzyme form may considerably differ from those observed with the more generally accepted substrates used in this study. A special problem also exists with respect to MO, because with many substrates distinct positions of attack exist. The present paper however suggests, as shown by the effects of various MO inhibitors (Fig. 1), that MO patterns are very similar in the same organ from different species, but vary more drastically in different organs of the same species.

The study also shows that rodent and human subcellular fractions from a given organ (lung or liver) had specific enzyme activities of a similar order of magnitude (differing by a factor <10) with one exception. Specific MO activity in human lung microsomes was two orders of magnitude lower than the lowest specific activity of this system measured in rodents. Other investigators also described very low activities in human lung

microsomes (53,67,68), but the contrast to rodents was smaller. In a separate report it will be shown that human lung preparations contain undialyzable MO inhibitors.[4] This may be related to large quantities of contaminants present in the available human lung samples. It is not known whether this inhibition is effective only in subcellular preparations or whether it occurs to a similar extent *in vivo*.

With the exception of human MO, species differences in the investigated enzyme activities were moderate. Yet even smaller differences between different animal strains have been shown to be sufficient for quite remarkable differences in toxic responses (69). Thus, the observed differences in the degree of similarity among the species are undoubtedly important. With respect to the investigated enzyme activities man and rat were most close to each other. Between these two species none of the investigated enzyme activities (with the exception of the above discussed lung MO) differed by a factor greater than 2. Toward the substrates tested mouse differed by substantially higher microsomal MO and cytosolic GST activities in both liver and lung and by a lower liver microsomal EH activity compared to man and rat. The hamster displayed high hepatic and pulmonary GST activity; among the four species tested it showed the highest liver MO and the highest lung EH activities. Thus the study shows that—with the possible exception of lung MO—rat is a good model for man: (a) with respect to the investigated enzyme activities, (b) with respect to liver–lung ratios of these enzyme activities, and (c) with respect to inhibition patterns of MO by different types of inhibitors.

SUMMARY

Activities of drug metabolizing enzymes were determined in subcellular fractions of lung biopsies from 12 human subjects and in liver biopsies from 15 other human subjects. Monooxygenase (MO) activity with 7-ethoxycoumarin as a substrate and epoxide hydrolase (EH) activity with benzo[*a*]pyrene 4,5-oxide as a substrate were measured in the microsomal fraction, glutathione S-transferase (GST) activity toward 2,4-dinitrochlorobenzene in the cytosolic fraction. MO activity was further characterized by the use of inhibitors known to act preferentially on different MO forms. To facilitate extrapolations from test results obtained in animals to man enzyme activities were also determined in corresponding fractions from commonly used laboratory animals, Sprague–Dawley rat, NMRI mouse, and Syrian golden hamster.

All investigated specific activities were lower in lung than in liver preparations by factors ranging from 2.4 to 7 in the mouse, 4 to 18 in rat and hamster, and 11 to 697 in man. The high ratio between liver and

[4] F. Oesch, H. Schmassmann, E. Ohnhaus, U. Althaus and J. Lorenz, in preparation.

lung activity in man occurred with MO and is due to an extremely low activity in human lung. It is not clear whether this low activity is predominantly due to low amounts of enzyme or to inhibitors known to be present in human lung preparations. With this exception of human lung MO, species differences in the investigated enzyme activities were moderate. Among the three rodent species, rat was most similar to man, with none of the investigated activities differing by a factor more than 2. The mouse differed from these two species by considerably higher MO and GST activities in both organs, and by a relatively low EH activity in liver for the investigated substrates, while the hamster displayed comparatively high lung GST and EH and liver GST and MO activities.

MO inhibition patterns by different *in vitro* inhibitors were similar in the same organs of different species, but differed in lung and liver. Standard concentrations of the diagnostic inhibitors led to preferential inhibition of lung MO by metyrapone and considerably less by tetrahydrofuran, while for liver MO the reverse was true. In both organs, the standard concentration of α-naphthoflavone had only very weak effects.

In conclusion, man and commonly used laboratory rodents are not grossly different with respect to the investigated enzyme activities with the possible exception of lung MO. For the substrates investigated, the rat represented clearly the best model for man among the studied animal species.

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